Protein Staining Procedures

Coomassie Blue Staining
Silver Nitrate Staining
Zinc Imidazole Negative Staining

This method was successful in our lab using prostate tissue and for our specific objectives. Investigators must be aware that they will need to tailor the following protocol for their own research objectives and tissue under study.

Coomassie Blue Staining

TIP: All washes and incubations are done with constant, gentle shaking.

1. Immerse gel in 50% ethanol/10% acetic acid for at least 1 hr.
2. Soak in 5% ethanol/5% acetic acid overnight or for a minimum of 2 hours.
3. Wash in diH_2O for 1 hr.
4. Add Gel-Code Blue Stain reagent (Pierce, #24592) for at least 3 hrs.
5. Wash in diH_2O twice, 15 min each.
6. Rinse in diH_2O for 1 hr.
7. Gels can be stored at 4°C.

Silver Nitrate Staining

A: Solutions:

1. Ammonical silver nitrate staining solution:

Prepare solutions A and B as described below and, after mixing together, make to the final volume. Dissolve each solution WELL by vortexing vigorously.

	Solution A			Solution B			
diH_2O (ml)	10 N NaOH (ml)	NH_4OH (ml)		Silver Nitrate (g)	diH_2O (ml)		Final Volume (ml)
21	0.2	1.3		1.5	4		100
31.5	0.3	1.95		2.25	6		150
105	1	6.5		7.5	20		500
210	2	13		15	40		1000
315	3	19.5		22.5	60		1500
420	4	26		30	80		2000

Add solution B to solution A slowly. A brown precipitate will appear then disappear. If disappearance of the brown precipitate is slow, add drops of NH4OH. Bring to final volume.

TIP: diH_2O should be high quality. Otherwise, silver nitrate will precipitate.

2. Developing solution (0.1% formaldehyde, 0.01% citric acid):

   100 µl formaldehyde and 0.01 g of citric acid /100 ml diH_2O

3. Stop solution (2% acetic acid):

   2 ml acetic acid plus 98 ml diH_2O

4. Destaining solution:

   Prepare separately:

   10 mM sodium thiosulfate pentahydrate
   (0.248 g/100 ml diH_2O)
   30 mM potassium ferricyanide
   (0.988 g/100 ml diH_2O)

   Mix both solutions together.

B: Procedure:

All washes and incubations are done with constant, gentle shaking.

1. Staining

1. Cut gels at the top/basic corner.
2. Immerse in 50% ethanol/10% acetic acid for at least 1 hr.
3. Soak in 5% ethanol/5% acetic acid overnight for a minimum of 2 hours to several days.
4. Wash in diH_2O for 1 hr.
5. Fix in 1% glutaraldhyde/0.5 M sodium acetate for 30-45 min.
6. Wash in diH_2O, three times for 15 min each.
7. Add Gel-Code Blue Stain reagent (Pierce, #24592) for at least 3 hrs.
8. Wash in diH_2O twice for 15 min each.
9. Wash in diH_2O for 1 hr.
10. Stain gel in ammonical silver nitrate solution for 1 hr.
11. Rinse gel three times for 5 min each in diH_2O.
12. Develop gel in developing solution for 5-10 min or until signal to noise is appropriate.
13. Stop development by adding 100-150 ml of the stop solution.
14. To re-stain, rinse gel in diH_2O three times for 15 min each, then repeat starting from the incubation with silver nitrate.

2. Destaining

1. If the gel is over-developed, it can be destained by adding the destaining solution to the gel for 30 sec-5 min.
2. The silver staining should fade away slowly.
3. At the right moment, scan the gel and save.
4. Otherwise, rinse extensively in diH_2O five or six times.
5. Repeat silver staining.

   Note: Staining intensity may be less than normal.

Zinc Imidazole Negative Staining

A: Solutions:

1. 1% sodium carbonate:

   1 g sodium carbonate/100 ml diH_2O

2. 200 mM imidazole/0.1% SDS:

   1.36 g imidazole and 0.1 g SDS per 100 ml diH_2O

3. 100 mM zinc acetate:

   2.2 g zinc acetate/100 ml diH_2O, filter through a 0.45 µm filter.

B: Procedure:

All washes and incubations are done with constant, gentle shaking.

1. Rinse gels in HPLC grade H_2O for 5 min.
2. Equilibrate gel in 1% sodium carbonate for 15 min.
3. Incubate gels in imidazole/SDS solution for 30 min.
4. Rinse gels in HPLC grade H_2O for 1 min.
5. Develop gels in zinc acetate solution for 1-5 min or until spots are well resolved.
6. Rinse gels in HPLC grade H_2O three times for 5 min each.
7. Spots can be cut out of the gels for sequencing, **or**
8. Gels can be:
   - stored at 4°C up to one week without significant protein diffusion
   - destained with 50 mM EDTA and washed for:
     - re-staining
     - protein transfer
     - silver staining